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United States Patent
Delcourt

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Cloning method by multiple-digestion, vectors for implementing same and applications

Abstract

The present invention pertains to a process for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a group of fragments by preparing an initial library of clones from the group of fragments using a vector containing no more than a predetermined number of known restriction sites, preferably 1-3 restriction sites, subjecting the initial library to at least 10, and preferably between 50 and 70 restriction enzymes different from those to which the vector is susceptible, to produce a group of monodigested libraries, screening the group of monodigested libraries for the target fragment to determine those restriction enzymes to which the target fragment is insensitive, and subjecting the initial library to substantially all of the restriction enzymes to which the target fragment is insensitive, to produce a multidigested library having an intact clone of the target nucleic acid fragment. The target fragment can then be separated, transfected, reproduced, and studied or sequenced.

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Parent Case Text

This is a continuation of Application No. PCT/FR98/02629, filed Dec. 4, 1998.

Claims

1. A process for isolating a target nucleic acid fragment having a known characteristic, from a number of fragments comprising said target nucleic acid fragment, said process comprising:

- a) identifying a target nucleic acid fragment having a known characteristic;
- b) providing a number of nucleic acid fragments of different characteristics, which are capable of containing one or more of said target nucleic acid fragment having a known characteristic;
- c) preparing a first initial library of clones from said number of fragments using a vector containing no more than a pre-determined number of known restriction sites;
- d) subjecting said first initial library to a plurality of restriction enzymes individually wherein digestion of said library is performed in parallel by each individual restriction enzyme, to produce a group of monodigested libraries which correspond in number to the number of plurality of restriction enzymes used;
- e) screening said group of monodigested libraries individually for said known characteristic to detect the presence of said target fragment, to thereby determine those restriction enzymes to which said target fragment is insensitive;
- f) preparing a second initial library which is substantially the same as the first initial library;
- g) producing a multidigested library by digesting said second initial library with all of the plurality of restriction enzymes to which said target fragment is insensitive as determined in step e, and obtaining said multidigested library which contains the target nucleic acid fragment; and

- h) isolating said target nucleic acid fragment from the multidigested libraries.
- 2. The process of claim 1 wherein said plurality of restriction enzymes comprises at least 10 restriction enzymes.
- 3. The process of claim 1 wherein said plurality of restriction enzymes comprises at least 50 restriction enzymes.
- 4. The process of claim 1 wherein said plurality of restriction enzymes comprises at least 70 restriction enzymes.
- 5. The process of claim 1 wherein said pre-determined number of known restriction sites is four.
- 6. The process of claim 1 wherein said pre-determined number of known restriction sites is three.
- 7. The process of claim 6 wherein at least one of said three sites is different from, and flanked by, said two remaining sites.
- 8. The process of claim 1 wherein said restriction enzymes have cleavage sites from 5 to 6 nucleotides in length.
- 9. The process of claim 1 including the further step of transforming and replicating said intact clone of the target nucleic acid fragment.
- 10. The process of claim 9 including the further step of isolating said intact clone.
- 11. The process of claim 1 comprising, after step b), the further step of transfecting said monodigested libraries in cellular hosts.
- 12. The process of claim 1 comprising the further step of verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment.
- 13. The process of claim 1 comprising the further step of verifying the presence of said target fragment in said multi-digested library by transforming said library and screening said transformed library for the presence of said target fragment.
- 14. The process of claim 1 wherein said number of fragments contains up to 108 fragments, each from about 00.1 kb to 5 kb in size.
- 15. A process for isolating a target nucleic acid fragment having a known characteristic, from a group of fragments comprising said target nucleic acid fragment, said process comprising:
 - a) identifying a target nucleic acid fragment having a known characteristic of interest;
 - b) providing a number of said nucleic acid fragments of different characteristics, which are capable of containing one or more of said target nucleic acid fragments having a known characteristic;
 - c) preparing first initial library of clones from said number of fragments using a vector containing no more than a pre-determined number of known restriction sites;
 - d) verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment;

- e) subjecting said first initial library to a plurality of restriction enzymes individually wherein digestion of said library is performed in parallel by each individual restriction enzyme, to produce a group of monodigested libraries which correspond in number to the number of plurality of restriction enzymes used;
- f) independently transfecting said monodigested libraries;
- g) screening said group of monodigested libraries for said known characteristic to detect the presence of intact target fragments, to thereby determine those restriction enzymes to which said target fragment is insensitive;
- h) preparing a second initial library which is substantially the same as said first initial library;
- i) subjecting said second initial library to all of said plurality of restriction enzymes to which said target fragment is insensitive as determined in step g, to produce a multidigested library having the target nucleic acid fragment;
- j) transforming said multidigested libraries; and
- k) isolating said target nucleic acid fragment.

16. The process of claim 15 wherein said restriction enzymes have cleavage sites from 5 nucleotides in length.

17. A process for isolating an intact clone of one a target nucleic acid fragment having a known characteristic, from a group of fragments, said method comprising:

- a) preparing first initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;
- b) subjecting said first initial library to a plurality of restriction enzymes individually wherein digestion of said library is performed in parallel by each individual restriction enzyme, to produce a group of monodigested libraries which correspond in number to the number of plurality of restriction enzymes used;
- c) transforming said monodigested libraries into bacteria;
- d) culturing said bacteria to produce digested libraries substantially free of cleaved products, cleaving each digested library to produce digestion products, depositing said products in an agarose gel well, migrating said products, transferring said products onto a membrane, hybridizing said transferred products with a probe, to thereby determine those restriction enzymes to which said target fragment is insensitive;
- e) preparing a second initial library which is the same as the first initial library;
- f) subjecting said second initial library to all of said plurality of restriction enzymes to which said target fragment is insensitive as determined in step d, to produce a multi-digested library having an intact clone of the target nucleic acid fragment; and
- g) isolating said target nucleic acid fragment.

18. A method for producing a group of monodigested libraries from a group of fragments, said method comprising:

- a) preparing an initial library of clones from said group of fragments using a vector containing no more than a pre-determined number of known restriction sites; and

b) subjecting said initial library to a plurality of restriction enzymes individually and in parallel, to produce a group of monodigested libraries corresponding in number to the number of restriction enzymes used.